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INFLUENCE OF GANGLIOSIDES GM_1 AND GD_{1a} ON STRUCTURAL AND THERMOTROPIC PROPERTIES OF SONICATED SMALL 1,2-DIPALMITOYL-L-α-PHOSPHATIDYLCHOLINE VESICLES

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Summary

The effect of the gangliosides GM_1 and GD_{1a} on the structure- and temperature-induced transition behavior of sonicated, small 1,2-dipalmitoylphosphatidylcholine (DPPC) vesicles has been studied by ^1H- and ^{13}C-NMR, laser light scattering and differential scanning microcalorimetry. It became apparent from comparison of the results obtained by the various methods that great care must be taken when the occurrence of phase transitions is postulated on the basis of methods other than high-sensitivity microcalorimetry. Detection of a temperature-dependent decrease in the half-width of methylene signals, which was reminiscent of a phase transition, was not paralleled by characteristic changes in heat capacity in the microcalorimetric experiments. Probing of the local mobility of the choline head groups in vesicles of mixed composition by ^1H-NMR and ^{13}C-NMR spin-lattice relaxation time (T_1) measurements suggested a decrease of mobility of the hydrophilic DPPC head groups in the presence of either ganglioside. The decreases in T_1 were identical for both gangliosides irrespective of the differences in the structure of the sugar moieties of GM_1 and GD_{1a}, whereas the heat capacity vs. temperature curves were rather different. Since also the average dimensions of pure DPPC vesicles and vesicles containing GM_1 or GD_{1a} were the same, the differences in temperature dependence of heat capacity in the presence of the gangliosides were attributed to differences in interaction of the hydrophobic parts of the molecules. The higher content of C_{20} sphingosines in GD_{1a} as compared to GM_1 was envisaged as a possible source for these differences. The presence of a physiological $CaCl_2$ concentration (2 mM) did not affect the transition behavior of GD_{1a}-containing vesicles to any detectable level, as monitored by calorimetry.

Introduction

Glycolipids are well known to be involved in a variety of cell surface phenomena. Sugar moieties are effective, highly specific, antigenic determinants [1,2] due to the large variety of stereoisomers; noteworthy is the group of blood antigens [3—5]. Glycolipids are also good candidates for specific cell-cell recognition and adhesion [6]. Changes in the glycolipid composition in membranes are often diagnostic of malfunction of cells. A high percentage of gangliosides, sialic acid-containing glycosphingolipids, is concurrent with various diseases, particularly of the nervous system [7,8]. Gangliosides serve as receptors for tetanus toxin [9—11], cholera toxin [12,13] and botulinus toxin [14]. Gangliosides also play an instrumental role in binding viruses, e.g., the sendai viruses [15] and the influenza virus [16,17]. A striking phenomenon is the changes in glycolipid concentration in membranes of tumor cells, like the alterations in polyoma-transformed baby hamster kidney cells [18,19] or in hepatoma 27 cells [20].

In comparison with the vast knowledge about biological functions of gangliosides, the understanding of their effects on the local membrane structure and on the physical properties of membrane constituents is rather limited. It is thus of importance to study the influence of gangliosides on well-characterized model systems in order to understand further the complex natural membranes. The present investigation reports ^{1}H- and ^{13}C-NMR, light scattering and microcalorimetric studies on transition properties of sonicated vesicles of DPPC in the presence of the purified gangliosides GM_1 and GD_{1a}.

Materials and Methods

Preparation and characterization of gangliosides. Crude ganglioside solutions were purchased from Koch Light. Purified gangliosides were obtained by using prepared analytical d.c. plates (20×20 cm^2, silica layer of 0.25 mm thickness) available from Merck, Darmstadt. 100 μl of the ganglioside mixture dissolved in $CHCl_3/CH_3OH/H_2O$ (12 : 7 : 1, v/v) were applied to the plates using a Desaga autoliner, and the chromatograms were developed in $CHCl_3/CH_3OH/H_2O$ (50 : 38 : 6, v/v). From 500 plates, 120 mg pure GM_1 and 170 mg pure GD_{1a} were obtained.

Each of the purified gangliosides showed only one spot on thin-layer chromatography plates when using *n*-propanol/water (7 : 3, v/v) or $CHCl_3/CH_3OH/H_2O$ (60 : 35 : 8, v/v) as solvents. For further identification, 0.5 mg of each ganglioside in 0.4 ml 2 mM $CaCl_2$ solution was incubated with 0.3 ml neuraminidase solution (0.05 M acetate buffer, pH 5.5) for 3 days at 37°C.

1 ml neuraminidase solution (Serva, Heidelberg, F.R.G.) produced 500 μg *N*-acetylneuraminic acid. Thin-layer chromatograms of the GM_1 + neuraminidase and the GD_{1a} + neuraminidase systems showed that GM_1 was not hydrolyzed by the enzyme and that one sialic acid molecule was removed from GD_{1a}. The monosialo ganglioside resulting from GD_{1a} upon this treatment had the same hydrophilic structure as GM_1 but had the sphingosine base composition of the parent ganglioside. In the thin-layer chromatogram, it showed one spot which could not be distinguished from that of GM_1. The fatty acid composition

TABLE I

FATTY ACID COMPOSITION OF GM_1 AND GD_{1a} AS DETERMINED BY GAS CHROMATOGRAFIC ANALYSIS OF THE METHYL ESTERS OF THE FATTY ACIDS [21]

A Varian 1400 gas chromatograph was used. The capillary column contained diethyleneglycolsuccinate. The carrier gas used was H_2 (0.6 atm) and the temperature was 150° C.

Ganglioside	Fatty acid (%)				
	C14 : 0	C16 : 0	C18 : 0	C18 : 3	C20 : 4
GM_1	1.23	4.70	90.09	2.86	1.11
GD_{1a}	1.13	4.58	90.00	3.01	1.27

of the gangliosides was determined by gas chromatography as described by Morrison and Smith [21]. Both gangliosides had essentially the same fatty acid composition as can be seen in Table I with stearic acid being the major component. The structure of the gangliosides is given in Fig. 1. DPPC was purchased from Koch-Light or Calbiochem, and was used without further purification [^{13}C]DPPC (90% labeled in one methyl group of the choline) was obtained from Lipid Specialities Inc., Boston, U.S.A. The following molecular weights were used for calculations: DPPC, 734; GM_1, 1500; GD_{1a}, 1808.

Preparation of vesicles. All vesicles were prepared using a Branson sonifier equipped with a microtip at power setting 6. The aqueous suspensions were sonicated for 30 min at 45—50°C under N_2. The aqueous suspensions were obtained from either DPPC solutions in chloroform or DPPC/ganglioside solutions in $CHCl_3/CH_3OH$ (3 : 1, v/v) after evaporation of the organic solvents and resuspension of the lipid film in H_2O or ^2H_2O, respectively. The sonicated vesicle preparation was subsequently centrifuged in a Sorvall centrifuge at 4000 rev./min for 30 min to remove Ti particles introduced by the sonication probe.

Phosphorus determination. Phospholipid concentration was calculated using phosphorus analysis. Aliquots of the vesicle preparation before and after the measurements were digested according to the method described by Ames and Durbin [22]. Phosphorus content was determined according to the procedure of Atkinson et al. [23].

NMR measurements. ^{1}H spin-latticce relaxation times, T_1, were determined following the method given by Vold et al. [24], using pulse sequences of 180°-τ-90°. ^{13}C T_1 values were obtained according to Freeman and Hill [25] employing the same pulse sequences.

The ^{1}H-Fourier Transform spectra were obtained using a Bruker WH 270 spectrometer, the Fourier Transform proton noise decoupled ^{13}C spectra were obtained employing a Bruker WH 90 spectrometer at 22.63 MHz. Both spectrometers were equipped with temperature control and were interfaced with Nicolet B-NC-12 computers. The samples were sealed under N_2 in glass tubes.

Light scattering measurements. The average radius, $\bar{r}$, of a vesicle population was obtained from the determination of the translational diffusion coefficient of the vesicles by laser light scattering, assuming validity of the Stokes' law for spherical particles. $\bar{r} = kT(6\pi\eta D)^{-1}$ where k is the Boltzman constant, T the thermodynamic temperature and η the viscosity of the solvent. D, the transla-

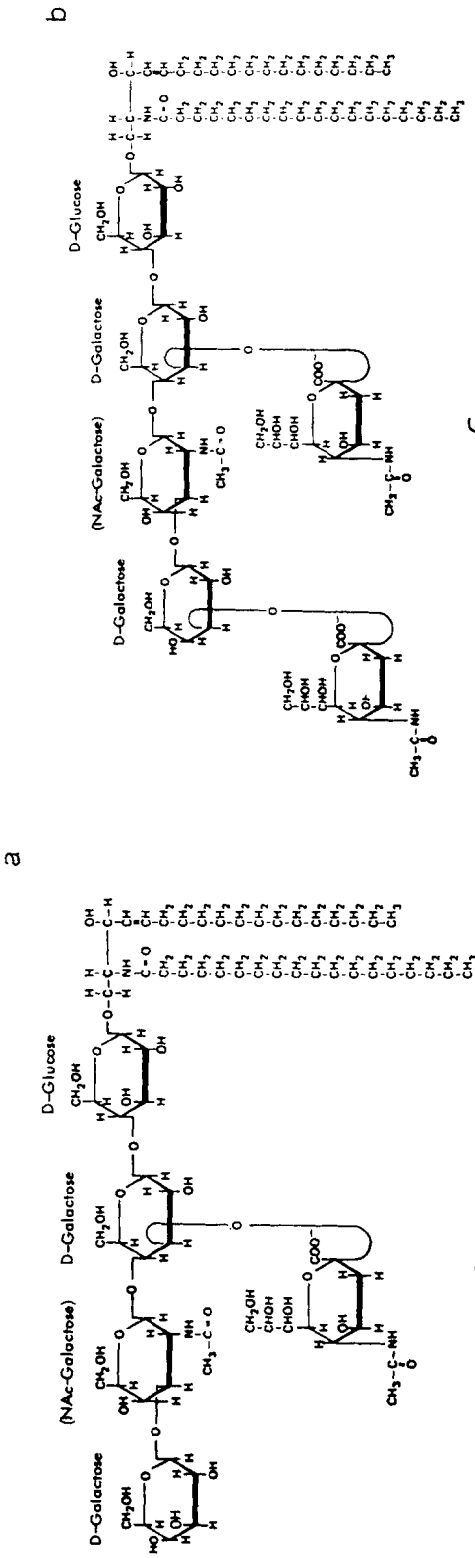

Fig. 1. Structure of GM_1 (a) and GD_{1a} (b). GD_{1a} contains two molecules of sialic acid, GM_1 only one. A further difference exists in the sphingosine base composition. GM_1 has 37.3% C_{20} sphingosine while GD_{1a} exhibits 56.5% [31]. The fatty acid chains consist predominantly of stearic acid (Table I).

tional diffusion coefficient, can be obtained from light scattering experiments. By plotting the light scattering half-line width at half maximum height (in Hz) as a function of $\sin^2(\frac{\theta}{2})$, one obtains from the slope of the resulting line the diffusion coefficient according to the equation

$$\Delta\nu_{1/2} = D\,\frac{8\pi n^2}{\lambda}\,\sin^2\left(\frac{\theta}{2}\right)$$

in which n is the index of refraction of the solution, λ is the wavelength of the incident light and θ is the angle between the incident and scattered beams. For these experiments, a Cilas Helium Neon laser was used (λ = 632.8 nm; 50 mW) in connection with a spectrum analyser (model of Dr. L. Boyer, Laboratoire de Spectrométrie Rayleigh-Brillouin, Montpellier, France) and a Hewlett Packard Computer model 4830.

Calorimetric measurements. The dependence on temperature of heat capacity, c_p, of vesicle suspensions was determined using a Privalov type DASM-1M differential scanning microcalorimeter [26]. The samples were loaded into the 1-ml sample cell after degassing under aspirator vacuum, and the reference cell was filled with quartz bidistilled water. Scans were made over the temperature range of approx. 5—80°C at a scan rate of 1 deg · min^{-1}. Power calibration was registered for each run. The van 't Hoff enthalpy, ΔH_{vH}, was calculated from the molar c_p vs. temperature curve employing the equation

$$\Delta H_{vH} = 4RT_m^2\,\frac{c_{p,max}}{\Delta H_{cal}}\,.$$

The apparent number of molecules in the cooperative unit, n, was obtained from the ratio $\Delta H_{vH}/\Delta H_{cal}$. T_m is the temperature of the maximum, $c_{p,max}$, of the c_p vs. temperature curve; R the gas constant and ΔH_{cal} the calorimetrically determined molar transition enthalpy.

Results

NMR measurements on pure gangliosides

^{1}H spectra of the two gangliosides GM_1 and GD_{1a} are shown in Fig. 2I and II. They are rather similar to known spectra of other lipids [27,28] in exhibiting signals of the methyl, methylene and *N*-acetyl groups. The difference in the number of *N*-acetylated sugars of GM_1 and GD_{1a} is reflected in the presence of two and three resonances, respectively, at approx. 2 ppm. The ^{1}H resonances of the carbohydrate rings are not resolved. Variation with temperature of the half-width of the resonance lines, $\Delta\nu_{1/2}$, of the methylene protons is shown in Fig. 3 for the temperature range 10—60°C. There is a decrease in half-width with increasing temperature for both GM_1 and GD_{1a}, reminiscent of similar findings with phospholipids. However, narrowing of the absorption lines of phospholipid suspensions occurs within approx. 1 degree, whereas the decrease in half-width of the resonance lines of gangliosides is spread over a temperature range of 20—30°C. The midpoint temperatures of these changes are 35°C for GM_1 and 25°C for GD_{1a}. It is noteworthy that the midpoint temperature of GD_{1a} is lower than that of GM_1 although the percentage of C_{20} sphingosines in GD_{1a} is

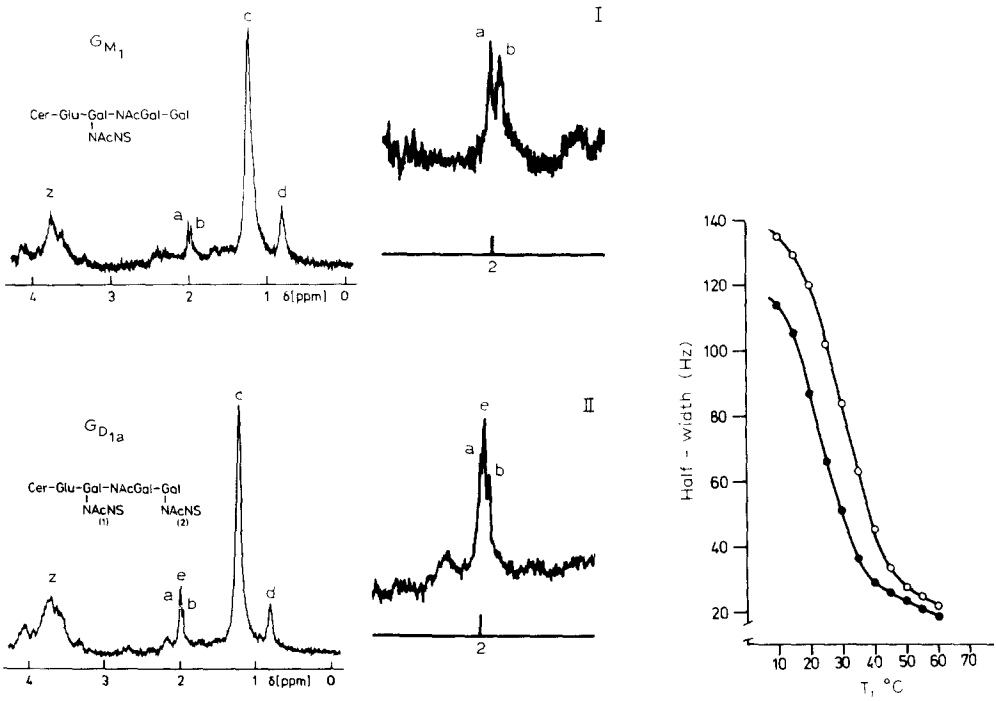

Fig. 2. (Left-hand figures). (I) 270 MHz ^{1}H-NMR spectrum of GM_1 in ^2H_2O. Ganglioside concentration = $8.5 \cdot 10^{-2}$ M; temperature = 25°C; 30 accumulations were made. The signals were assigned in the following manner [29,30]: (a) *N*-acetyl signal of neuraminic acid; (b) *N*-acetyl signal of *N*-acetylgalactosamine; (c) $-(CH_2)_n-$ signal; (d) $-CH_3$ signal; (z) signal of the sugar ring hydrogens. (II) 270 MHz ^{1}H-NMR spectrum of GD_{1a} in ^2H_2O. Ganglioside concentration = $8.5 \cdot 10^{-2}$ M; temperature = 25°C; 30 accumulations were made. (a) *N*-acetyl signal of neuraminic acid (1); (b) *N*-acetyl signal of *N*-acetylgalactosamine; (c) $-(CH_2)_n-$ signal; (d) $-CH_3$ signal; (e) *N*-acetyl signal of neuraminic acid (2); (z) signal of the sugar ring hydrogens.

Fig. 3. (Right-hand figure). Variation with temperature of the half-widths (Hz) of the methylene signals of GM_1 and GD_{1a} vesicles in ^2H_2O as obtained from 270 MHz ^{1}H-NMR spectra. Ganglioside concentration = $8.5 \cdot 10^{-2}$ M; 30 accumulations were made at each temperature. (○) GM_1; (●) GD_{1a}.

56.5% while that in GM_1 is only 37.3% [21]. The increase in C_{20} chains relative to that of the C_{18} chains would lead one to expect a higher midpoint temperature for GD_{1a} than for GM_1 if the hydrophobic part of the molecule played a similarly important role for gangliosides as for phospholipids. However, the experimental finding that the midpoint temperature is lower for GD_{1a} than for GM_1 is suggestive of a significant role of the hydrophilic head groups in the organization of the micelles of pure gangliosides. This conclusion gains support from a comparison of the average number of ganglioside molecules per vesicle [29], which is 225 ± 20 for GM_1 and 165 ± 15 for GD_{1a}. It is the second sialic acid molecule present in GD_{1a} which appears to be responsible for these differences, since the ganglioside, which results from GD_{1a} after removal of the extra sialic acid by action of neuraminidase, exhibits practically the same number of molecules (240 ± 15) in the micelle as GM_1. Table II shows a comparison of T_1 values for GM_1 and GD_{1a} suspensions determined at 52°C in ^2H_2O. The lower

TABLE II

^1H SPIN-LATTICE RELAXATION TIMES (T_1) OBTAINED FOR SONICATED GM_1 AND GD_{1a} SUSPENSIONS IN ^2H_2O at 52°C

The ganglioside concentration was $8.5 \cdot 10^{-2}$ M. 270 MHz ^1H-NMR spectra were recorded 30 accumulations $(180°\text{-}\tau\text{-}90°\text{-}t)_n$; t = 11 s.

	^1H T_1 values (ms)	
	GM_1	GD_{1a}
N-acetylgalactosamine	950 ± 60	1250 ± 100
N-acetylneuraminic acid (1)	1050 ± 80	1350 ± 100
N-acetylneuraminic acid (2)		1450 ± 120
$-(CH_2)_n-$	600 ± 40	650 ± 50
$-CH_3$	900 ± 50	1070 ± 85

packing density of the hydrophobic side chains in GD_{1a}, suggested by the smaller number of molecules per micelle, is also apparent in the higher T_1 values obtained for GD_{1a}. The T_1 values for GD_{1a} are consistently higher than those obtained for GM_1. On the basis of current interpretations of spin-lattice relaxation times in phospholipid suspensions [30—32], this finding can be considered to suggest a lower rate of energy dissipation in GD_{1a} micelles as a result of the lower packing density.

NMR and Rayleigh laser light scattering studies on phospholipid/ganglioside mixtures

Table III summarizes spin-lattice relaxation times for the $-CH_3$ protons of the choline groups of pure DPPC vesicles, for DPPC vesicles containing GD_{1a} and for DPPC vesicles incorporated with GM_1. The DPPC/ganglioside ratio was 10 : 1 in each case, and the measurements were performed at 57°C.

It can be seen that both the gangliosides decrease the T_1 values in the same manner irrespective of their structural differences. This result indicates that the presence of the gangliosides in the DPPC vesicle bilayer reduces the mobility of

TABLE III

^1H SPIN-LATTICE RELAXATION TIMES (T_1) TRANSLATIONAL DIFFUSION COEFFICIENTS (D) AND AVERAGE RADII ($\bar{r}$) OF SONICATED SMALL DPPPC VESICLES AND DPPC VESICLES CONTAINING GM_1 OR GD_{1a}

DDPC concentration was 10^{-2} M. The molar ratio of ganglioside/DPPC was 1 : 10. T_1 values were derived from 270 MHz ^1H-NMR spectra of the methyl groups of the choline moieties of DPPC at 57°C, pH approx. 5.5—6.0 in ^2H_2O. 300 accumulations $(180°\text{-}\tau\text{-}90°\text{-}t)_n$; t = 6 s. The light scattering experiments were carried out at 25°C.

	^1H T_1 values (ms)	$D \times 10^8$ ($cm^2 \cdot s^{-1}$)	$\bar{r}$ (nm)
DPPC	680 ± 35	8 ± 1	27.0 ± 3.0
DPPC + GM_1	600 ± 30	9 ± 1	25.0 ± 2.5
DPPC + GD_{1a}	600 ± 25	9 ± 1	24.5 ± 2.5

protons of the choline head groups. It is well known that changes in size of the vesicles could result in alterations of the NMR spectra as well as the relaxation times. Therefore, Rayleigh light scattering experiments were performed to determine the influence of incorporation of gangliosides on the dimensions of the vesicles. As shown in Table III, hardly any change in the average radius of the vesicles can be detected when vesicles are formed from DPPC/ganglioside mixtures instead of DPPC alone. Unfortunately, this result does not allow us to conclude that size changes do not play any role, because all that light scattering allows us to calculate is the average radius of the vesicles; occurrence of variations in the distribution of vesicle radii cannot be eliminated.

^{13}C-NMR studies

^{13}C spin-lattice relaxation time depends on intramolecular dipole interactions. It is practically independent of vesicle size, if completely proton noise decoupled ^{13}C spectra are recorded. This has been demonstrated by the detection of identical ^{13}C T_1 values for large liposomes and small vesicles [34,35]. Therefore, a decrease in T_1 values of ^{13}C-labeled choline methyl head groups of DPPC in the presence of gangliosides can be interpreted unequivocally as a decrease in mobility of the $-\overset{(+)}{N}(CH_3)_3$ moiety.

Table IV gives the results of ^{13}C relaxation measurements on DPPC + GM_1 vesicles and DPPC + GD_{1a} vesicles. In the vesicles containing the gangliosides, the molar ratio of DPPC/ganglioside was 8 : 1, the temperatures at which the measurements were performed were 37°C and 54°C, respectively.

Inspection of the table shows a decrease in ^{13}C T_1 values as a result of incorporation of GM_1 and GD_{1a} into DPPC vesicles at both temperatures. This finding substantiates the conclusion, previously drawn on the basis of ^{1}H T_1 relaxation time measurements, that gangliosides decrease the mobility of the choline head groups in DPPC vesicles. The increase in ^{13}C T_1 values with increasing temperature reflects the usual rise in mobility above the transition temperature.

Calorimetric measurements

Studies on pure gangliosides. Fig. 4 illustrates the results of apparent heat capacity measurements on pure sonicated DPPC vesicles and GM_1 and GD_{1a}

TABLE IV

^{13}C SPIN-LATTICE RELAXATION TIMES (T_1) OF SONICATED SMALL DPPC VESICLES AND DPPC VESICLES CONTAINING GM_1 OR GD_{1a}

The ^{13}C T_1 values were derived from proton noise decoupled ^{13}C-NMR spectra of DPPC vesicles labelled in one of the methyl groups of the choline moiety. The DPPC concentration was $7.0 \cdot 10^{-2}$ M, the molar ratio of DPPC/ganglioside was 8 : 1, the temperature was 37°C or 54°C. 1000 accumulations $(180°\text{-}\tau\text{-}90°\text{-}t)_n$; t = 5 s.

	^{13}C T_1 values (ms)	
	37°C	54°C
DPPC vesicles	435 ± 20	745 ± 35
DPPC + GM_1 vesicles	320 ± 20	625 ± 30
DPPC + GD_{1a} vesicles	320 ± 20	625 ± 30

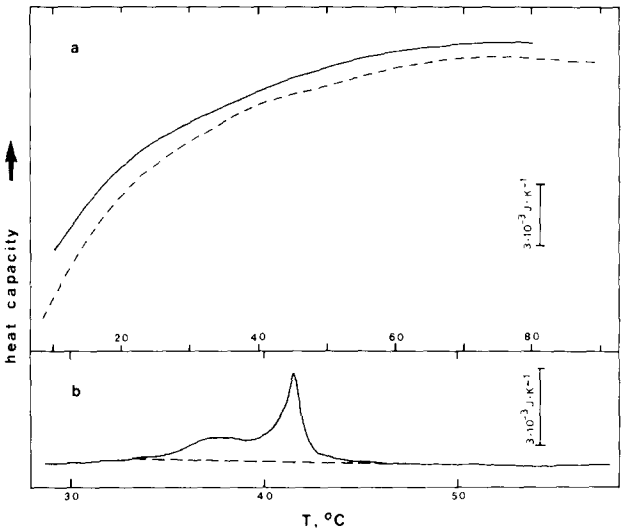

Fig. 4. Variation with temperature of the apparent heat capacity of sonicated small DPPC vesicles and sonicated GM_1 and GD_{1a} micelles in H_2O. (a) (———) GD_{1a}, 13.5 mg/ml; (- - - - -) GM_1, 15.5 mg/ml. (b) DPPC, 0.275 mg/ml; ΔH = 5.7 kcal/mol (23.7 kJ/mol).

micelle suspensions as a function of temperature. The heat capacity vs. temperature curves of pure sonicated DPPC vesicles (Fig. 4b) shows two distinct heat capacity maxima; a broad maximum at about 37°C and a relatively sharp maximum at 41.6°C. The overall enthalpy change associated with the transition is considerably lower than that observed for unsonicated multilamellar DPPC vesicles, and the excess heat capacity maximum at 41.6°C, although indicative of the presence of multilamellar structure, is lower than that determined for unsonicated vesicles. The scans of sonicated suspensions of GM_1 and GD_{1a} (Fig. 4a) exhibit no sign of a cooperative phase transition in the temperature range of 10—90°C. There is a gradual increase in heat capacity with increasing temperature but no peak which could be ascribed to the existence of a phase transition, although concentrations were as high as 15 mg/ml, i.e., well beyond critical micelle concentration. This result is in agreement with the absence of transitions in scans on pure unsonicated suspensions of GM_1 (4 mg/ml) reported by Sillerud et al. [35].

Measurements on GM_1/DPPC mixtures. Transition curves of various GM_1/DPPC mixtures are presented in Fig. 5. It is apparent that gangliosides perturb vesicles dramatically at molar ratios of 4.7 : 1 of DPPC/GM_1 (x_g = 0.18), as indicated by the broadening of the range over which the temperature-induced transition occurs. While in pure DPPC vesicles the usual two transitions are observed between 35 and 45°C, vesicles containing GM_1 exhibit one or two transitions, depending on the GM_1 content, between approx. 20 and 50°C. At high lipid to GM_1 ratios, the heat capacity curve shows only one transition which appears to be fairly symmetric and has a temperature at its maximum slightly below that found for the transition characteristic of lamellar structures (41.5°C). Increase of the GM_1 content leads to the appearance of a shoulder at around 32—33°C, which becomes progressively pronounced concomitant with

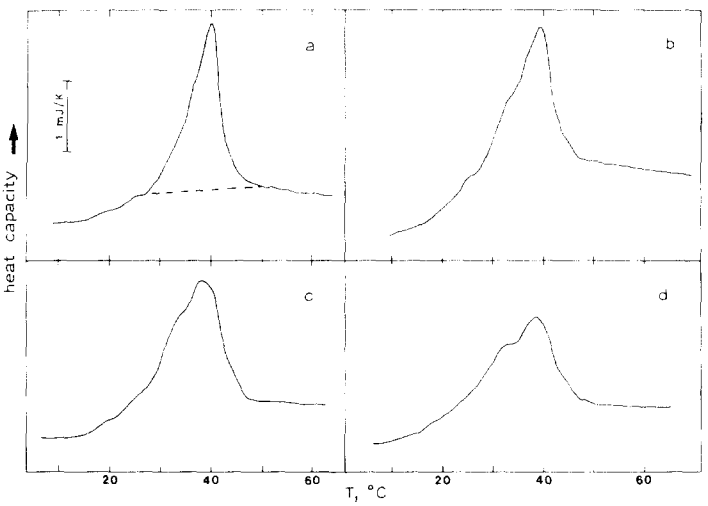

Fig. 5. Dependence on temperature of apparent heat capacity of sonicated small vesicles of GM_1/DPPC mixtures in H_2O. x_g refers to the mole fraction of ganglioside. (a) DPPC; 0.513 mg ($6.99 \cdot 10^{-7}$ mol); GM_1, 0.223 mg ($1.487 \cdot 10^{-7}$ mol); $x_g = 0.18$. The molar enthalpy, ΔH, has been evaluated using the dashed baseline. (b) DPPC, 0.511 mg ($6.955 \cdot 10^{-7}$ mol); GM_1, 0.255 mg ($1.7 \cdot 10^{-7}$ mol); $x_g = 0.2$. (c) DPPC, 0.459 mg ($6.247 \cdot 10^{-7}$ mol); GM_1, 0.377 mg ($2.513 \cdot 10^{-7}$ mol); $x_g = 0.827$. (d) DPPC, 0.2865 mg ($3.903 \cdot 10^{-7}$ mol); GM_1, 0.318 mg ($2.12 \cdot 10^{-7}$ mol); $x_g = 0.35$.

the increase in the GM_1 content. At a molar ratio of 1 : 1.8 of GM_1/DPPC (x_g = 0.35), the shoulder is clearly suggestive of a second transition appearing before the main transition. Close inspection of Fig. 5d reveals a deviation from a smooth curve at approx. 43°C which might indicate yet another transition.

Measurements on GD_{1a}/DPPC mixtures. Fig. 6 shows the scans of three suc-

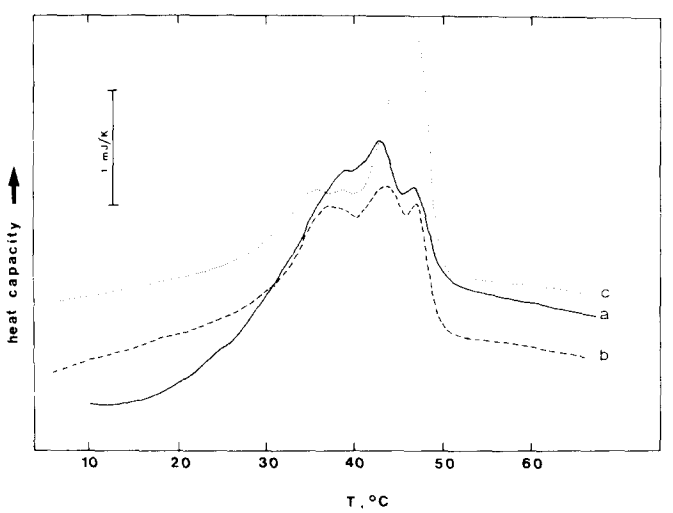

Fig. 6. Three successive determinations of apparent heat capacity as a function of temperature on the same sample. The sample was 1 ml of sonicated vesicles of a DPPC/GD_{1a} mixture in H_2O. DPPC, 0.456 mg; GD_{1a}, 0.215 mg; $x_g = 0.16$. (a) Scan 1 h after sample preparation; (b) scan 3 h after first measurement; (c) scan 10 h after first measurement (sample was kept for 3 h at 1°C after the second scan).

cessive measurements on the same sample. The first c_p vs. temperature trace (Fig. 6, curve a) obtained approx. 1 h after vesicle preparation exhibits three c_p maxima at roughly 39, 43 and 47°C. The second heating (Fig. 6, curve b) leads to a curve which still shows similar shape but shifted temperatures of the c_p maxima. In the third heating (Fig. 6, curve c) after keeping the sample at 1°C for 3 h, dramatic changes occurred in that the previously major peak at 43°C had been reduced to a shoulder at 45°C on a now dominant peak having a temperature of the maximum at 47°C. We take this series of measurements as evidence for the time-dependent merging of small monolayer vesicles. This conclusion is in accordance with the known instability of small vesicles [36—39].

Figs. 7 and 8 give additional calorimetric first heating scans of mixtures of DPPC with GD_{1a}. In comparison with the transition peaks of mixtures of DPPC with GM_1, the two sialic acid-containing GD_{1a} shows more complex transition patterns when mixed with DPPC than does GM_1. As was evident already in Fig. 6, even at low ganglioside to DPPC ratios, the c_p vs. temperature curve indicates the occurrence of three transitions. The high temperature transition has a c_p maximum, depending on the GD_{1a} content, between 46.8 and 48.2°C (Fig. 7, curves a—c). At a molar ratio of 2.7 : 1 of DPPC/GD_{1a} (x_g = 0.27), this peak is absent (Fig. 8, curve a) and on further increasing the GD_{1a} concentration up to approx. 1 : 1 molar ratio (Fig. 8, curve b), the c_p curve does not indicate a high temperature transition. The temperatures of the c_p maxima of the transition in the middle assume the following values as a function of the mole fraction: x_g = 0.16, T_m = 43°C (Fig. 7, curve a); x_g = 0.18, T_m = 41.7°C (Fig. 7, curve b); x_g = 0.25, T_m = 41.4°C (Fig. 7, curve c); x_g = 0.27, T_m = 43°C (Fig. 8, curve a); x_g = 0.47, T_m = 41.4°C (Fig. 8, curve b). Due to the broadness of the transition curves, it is difficult to assign temperatures to the maximum of the low temperature transition. At higher mole ratios than x_g = 0.27, only a small shoulder at the low temperature side of the transition peak is reminiscent

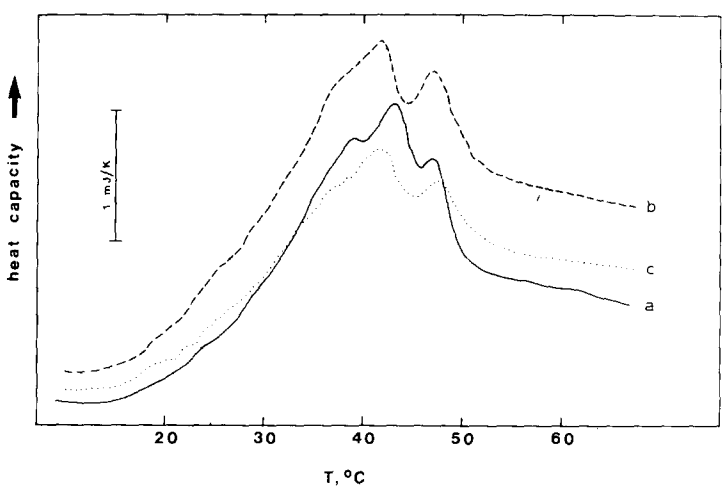

Fig. 7. Variation with temperature of apparent heat capacity of sonicated small vesicles of DPPC/GD_{1a} mixtures in H_2O. Sample volume was 1 ml. (a) DPPC, 0.465 mg ($6.212 \cdot 10^{-7}$ mol); GD_{1a}, 0.215 mg ($1.189 \cdot 10^{-7}$ mol); x_g = 0.16. (b) DPPC, 0.481 mg ($6.533 \cdot 10^{-7}$ mol); GD_{1a}, 0.266 mg ($1.471 \cdot 10^{-7}$ mol); x_g = 0.18. (c) DPPC, 0.44 mg ($6.049 \cdot 10^{-7}$ mol); GD_{1a}, 0.358 mg ($1.980 \cdot 10^{-7}$ mol); x_g = 0.25.

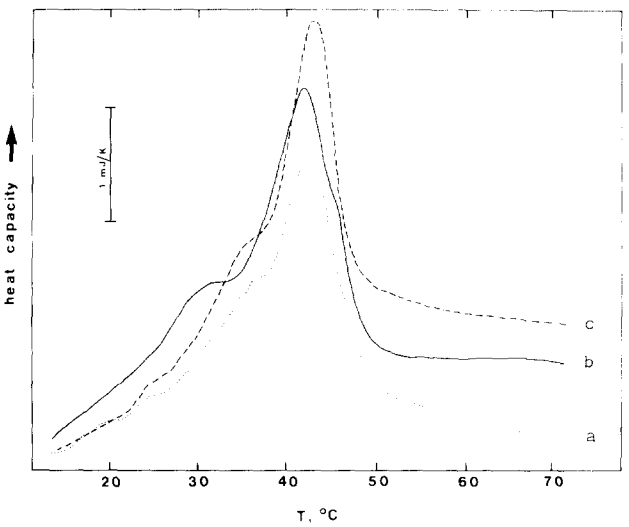

Fig. 8. Variation with temperature of apparent heat capacity of sonicated small vesicles of DPPC/GD_{1a} mixtures in H_2O. Sample volume was 1 ml. (a) DPPC, 0.515 mg ($7.016 \cdot 10^{-7}$ mol); GD_{1a}, 0.465 mg ($2.57 \cdot 10^{-7}$ mol); $x_g = 0.27$. (b) DPPC, 0.521 mg ($7.098 \cdot 10^{-7}$ mol); GD_{1a}, 1.12 mg ($6.20 \cdot 10^{-7}$ mol); $x_g = 0.47$. (c) DPPC, 0.444 mg ($6.05 \cdot 10^{-7}$ mol); GD_{1a}, 0.465 mg ($2.57 \cdot 10^{-7}$ mol); $x_g = 0.30$. This measurement was performed on vesicles prepared in the presence of 2 mM $CaCl_2$. The reference cell contained 2 mM $CaCl_2$ solution.

of the low temperature transition. A calorimetric scan of DPPC/GD_{1a} vesicles ($x_g = 0.30$) obtained in the presence of 2 mM $CaCl_2$ in the buffer is shown in Fig. 8, curve c. It appears to be not very different from the scan obtained without $CaCl_2$ in solution at comparable mole fractions of GD_{1a}, thus demonstrating the absence of the influence of Ca^{2+} on the transition properties of these vesicles.

Discussion

NMR and calorimetric studies on pure ganglioside suspensions

If the ^{1}H-NMR studies are compared with the calorimetric measurements, an apparent discrepancy becomes evident. While the variation with temperature of the half-width of the methylene signals of GM_1 and GD_{1a} suspensions appears to imply that a broad transition occurs between approx. 15 and 50°C with either compound (Fig. 3), the heat capacity vs. temperature curves (Fig. 4a) give no indication of a phase transition. There is a gradual increase in the heat capacity leveling off at about 60°C, indicative of heat-consuming processes occurring in solution. These changes are, however, not comparable to the structural changes involved in the reversible phase transitions found in phospholipids. Any change in the distribution between two states which adsorbs heat is associated with a maximum in the calorimetric c_p vs. temperature curve. Such a variation with temperature of the heat capacity of the ganglioside suspensions is not seen in the c_p curve. Thus, one has to conclude that the gradual increase in heat capacity cannot result from a phase transition analogous to

that found in phospholipid suspensions but that it reflects an increase in the number of energy-absorbing modes due, perhaps, to expansion of the micelles with increasing temperature [40]. At very high ganglioside concentrations (64.6 and 95.2 wt. %), two broad transitions have been observed, however, by differential thermal analysis studies [41]. The temperature of the c_p maxima varied with the water content, the low temperature transition occurring between 25 and 30°C, the high temperature transition between 43 and 47°C [41].

The interpretation given in Results for the lower midpoint temperature of GD_{1a} relative to GM_1, when the decrease of the half-width of the ^{1}H-NMR resonance lines with temperature is determined, is not unambiguous, although it is consistent with the number of gangliosides per micelle. It may well be that the different intramolecular distribution of the C_{20} and C_{18} chains in GM_1 and GD_{1a} also contribute to the lower midpoint temperature of GD_{1a}. There is, however, no published evidence as yet, but a personal communication (Sturtevant, J.M.) establishes that, for example, differences in chain lengths of fatty acids in the same phospholipid molecule in the calorimetric transition show properties which are distinct from those of mixtures of different phospholipids of intrinsically homogeneous fatty acid chain lengths. The different ratios between the C_{18} and C_{20} chains of GD_{1a} and GM_1 gangliosides may thus also be responsible for the perhaps surprising lower midpoint temperature for GD_{1a} micelles.

The apparent discrepancy between the NMR and calorimetric results is the second example of measurements in which phase changes of conformational transitions have been postulated on the basis of NMR or fluorescence measurements that could not be substantiated by microcalorimetric studies [42]. Since differential scanning calorimetry is certainly the most direct means for observing thermally induced changes, any failure to detect the phase transition in a calorimeter of high sensitivity casts serious doubts on its existence.

NMR and calorimetric studies on DPPC/ganglioside mixtures

A characteristic feature observed with all calorimetric measurements on vesicles of mixed composition is the extreme broadness of the transition curves. Due to the difficulties in assigning baselines and the arbitrariness involved in resolving the overall transition curves into subtransitions, calculation of values for cooperative units and enthalpies was not attempted for transitions other than that shown in Fig. 5a. The values of ΔH = 7.5 kcal/mol DPPC and of the apparent number (n) of molecules in the cooperative unit, n = 14, for this transition reflect a relatively minor interference of the presence of GM_1 with the energetics of the DPPC transition but a drastic influence on its cooperativity. This result is in agreement with the findings of Sillerud et al. [35], who also observed a dramatic decrease in the size of the cooperative unit of multilamellar dispersions of DPPC in the presence of GM_1.

There is no indication in our measurements on sonicated mixtures of the existence of a high temperature transition similar to that obtained by Sillerud et al. [35] with DPPC/GM_1 mixtures of high ganglioside content. Increasing the mole fraction of GM_1 induces a low temperature transition as shown in Fig. 5b–d. The differences between the results are very likely due to the fact that

our studies were done using sonicated small vesicles while those done by Sillerud et al. [35] refer to multilamellar large vesicles and bilayers.

It is remarkable that both the ^1H T_1 and ^{13}C T_1 measurements show that the effect of both the gangliosides on the relaxation properties of the choline groups is the same, irrespective of the presence of a second charged *N*-acetylneuraminic acid group in the hydrophilic part of the GD_{1a} molecule. Below and above the transition temperature, the relative decrease in the T_1 values is comparable, suggesting a similar reduction in mobility of the choline head groups at both temperatures. The identical effects of GM_1 and GD_{1a} on T_1 are surprising in view of the large differences, which are apparent in the heat capacity curves. Particularly, at comparably low mole fractions ($x_g < 0.25$), GD_{1a} seems to perturb the membrane structure more severely than GM_1. A higher GD_{1a} content ($x_g > 0.25$) leads to transition properties of the vesicles, which resemble more closely those exhibited by GM_1/DPPC mixtures, except that the temperature of the c_p maximum of the high temperature transition is higher in the presence of GD_{1a}.

If the identity of the decrease in T_1 of the choline head groups caused by the introduction of either GM_1 or GD_{1a} into DPPC can be assumed to reflect formation of a similar arrangement of the hydrophilic head groups in GM_1- and GD_{1a}-containing vesicles, the differences in the c_p curves must arise from differferences in the interaction between the hydrophobic moieties, since the average dimensions of the vesicles are also the same (Table III). A possible explanation for this discrepancy is the higher content of C_{20} sphingosine chains in GD_{1a} relative to GM_1.

Since incorporation of C_{20} chains into the hydrophobic C_{16} lattice of DPPC is likely to perturb the homogeneous structure of the vesicles, the effect of GD_{1a} should be larger than that of GM_1 at low mole fractions. At high mole fractions of gangliosides, the relative differences between them become less important. Fig. 8, curve c shows a transition curve obtained with GD_{1a} + DPPC vesicles ($x_g = 0.30$) in the presence of 2 mM $CaCl_2$. The curve resembles the two others shown in Fig. 8 and does not display any particular feature. Results of spin label experiments [43] have led to the assumption that the presence of divalent cations in solution at physiological concentrations resulted in cross-linking and condensing of ganglioside head groups by complexing sialic acid carboxyl residues. It can be inferred from the identity of the calorimetric curves of the GD_{1a} + DPPC vesicles in the presence or absence of Ca^{2+} in solution, that such clustering of the sugar moieties of the gangliosides does not affect the transition properties monitored by calorimetry. This conclusion is consistent with the interpretation given before to explain the differences between the T_1 measurements and the calorimetric studies. Apparently, in both cases discrepancies can be resolved by the assumption that changes in mobility of the head groups do not exert a detectable influence on the transition properties of the alkyl chains, the structural changes of which provide the major contribution to energetics of phospholipid phase transitions.

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